

Available online on 15.03.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open  Access

Research Article

Preparation and characterization of azathioprine microspheres for colon specific delivery

Rinku Gonekar, Mohan Lal Kori*

Vedica college of B. Pharmacy, RKDF University, Gandhi Nagar, 462033, Bhopal (M.P.) - India

ABSTRACT

The objective of the present study is to develop colon targeted drug delivery system using dextrin (polysaccharide) as a carrier for Azathioprine. Microspheres containing azathioprine, dextrin and various excipients were prepared by solvent evaporation technique. The prepared microsphere were evaluated by different methods parameters like particle size, drug entrapment efficiency, percentage yield, shape and surface morphology and in vitro drug release study. Drug release profile was evaluated in simulated gastric, intestinal fluid and simulated colonic fluid. Best formulation was decided on the basis drug release profile in simulated gastric, intestinal fluid and simulated colonic fluid. In dextrin based microspheres, dextrin as a carrier was found to be suitable for targeting of Azathioprine for local action in the site of colon. Dextrin microspheres released 95-99% of azathioprine in simulated colonic fluid with 4% human fecal matter solution. The results of in-vitro studies of the azathioprine microspheres indicate that for colon targeting dextrin are suitable carriers to deliver the drug specifically in the colonic region. Dextrin based azathioprine microspheres showed no significance change in particle size and % residual upon storage at $5 \pm 3^\circ\text{C}$, $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$ (room temperature) and $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$ humidity for three months.

Keywords: azathioprine, microsphere, dextrin, colon specific drug delivery.**Article Info:** Received 25 Jan 2019; Review Completed 28 Feb 2019; Accepted 02 March 2019; Available online 15 March 2019

Cite this article as:

Gonekar R, Kori ML, Preparation and characterization of azathioprine microspheres for colon specific delivery, Journal of Drug Delivery and Therapeutics. 2019; 9(2):97-101 <http://dx.doi.org/10.22270/jddt.v9i2.2519>

*Address for Correspondence:

Mohan Lal Kori, Vedica college of B. Pharmacy, RKDF University, Gandhi Nagar, 462033, Bhopal (M.P.) - India

1. INTRODUCTION

Since from last decade a novel oral colon-specific drug delivery system (CDDS) has been developing as one of the site-specific drug delivery systems. This delivery system, by means of combination of one or more controlled release mechanisms, this system hardly allow to releases drug in the upper part of the gastrointestinal (GI) tract, but rapidly releases drug in the site of colon following oral administration^{1, 2, 3}. CDDS is convenient for treating localized colonic diseases, i.e. ulcerative colitis, Crohn's disease and constipation etc., CDDS, also selectively deliver drug to the colon, but not to the upper GI tract¹. Colon is referred to as the optimal absorption site for protein and polypeptide after oral administration, because of the existence of relatively low proteolytic enzyme activities and quite long transit time in the colon. CDDS would be advantageous when a delay in absorption is desirable from a therapeutically point of view, as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis^{1, 2, 4}.

There were currently a few strategies to achieve colonic specificity, such as use of pH sensitive polymers and pressure-controlled CDDS. The aim of this study was to

explore the feasibility of the colonic microorganism to develop CDDS by using azathioprine and budesonide as a model drugs^{1, 5}.

Polysaccharides, the monosaccharide polymer retains their integrity because they are resistant to the digestive action of gastrointestinal enzymes of stomach. The matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine but once they reach in the colon, they are acted upon by the bacterial polysaccharides and results in the degradation of the matrices. A large number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans, dextrin and locust bean gum have been investigated for their use in colon targeted drug delivery systems. The most important fact in the development of polysaccharide derivatives for colon targeted drug delivery is the selection of a suitable biodegradable polysaccharide. As these polysaccharides are usually soluble in water, they must be made water insoluble by cross linking or hydrophobic derivatisation, very important is an optimal proportional of the hydrophobic and hydrophilic parts respectively and the number of free hydroxyl groups in the polymeric molecule. The objective of the present study is to develop colon targeted drug delivery

system by using dextrin as a carrier for Azathioprine. CDDS is also selectively delivered drug to colon but not to the upper tract ^{6,7}.

2. MATERIAL AND METHODS

Azathioprine was obtained from Sun Pharmaceuticals Limited, Mumbai. Dextrin and Span 80 was purchased from Sigma Aldrich Pvt Ltd. All other ingredients, solvents used for assay were of analytical grade.

Method

The azathioprine microspheres were prepared by solvent evaporation method. The drug (azathioprine) and dextrin (1%, 5%, 10% & 20% w/v) were dissolved in

dichloromethane. This solution was dispersed in 100 ml of liquid paraffin light containing various concentration of Span 80 in a 250 ml beaker. The dispersion was stirred at 200 rpm for 30 min. After the stirring time, microspheres were centrifuged, washed several times with n-hexane, ether and finally with acetone. The microspheres were dried at 50°C and stored in desiccator ⁸. Then optimization done on the basis of drug concentration, stirring time, surfactant concentration, temperature, and polymer concentration shown in table 1. Total 19 batches were prepared for azathioprine microspheres. In which 4 formulations were prepared with different drug polymer ratio. These 4 formulation of azathioprine microspheres further evaluated.

Table 1: Optimization of Azathioprine microspheres

Formulation code	Drug in Mg	Span 80 (%w/w)	Stirring speed (rpm)	Temperature	Dextrin (%w/v)	Mean size (µm)	% drug entrapment efficiency
F1 A	25	0.5	400	37 °	5	83±2.8	94.2±1.4
F2 A	50	-	-	-	-	85±1.9	83.5±1.8
F3 A	75	-	-	-	-	87±1.8	78.8±1.2
F4 A	100	-	-	-	-	88±1.2	74.3±2.8
F5 A	25	0.5	400	37 °	5	75.8±1.2	92.8±1.4
F6 A	-	0.75	-	-	-	73.9±0.9	74.8±1.2
F7 A	-	1.0	-	-	-	71.2±1.2	72.5±2.6
F8 A	-	1.25	-	-	-	70.2±1.6	68.7±2.2
F9 A	25	0.5	200	37 °	5	80.2±2.2	92.6±1.4
F10 A	-	-	300	-	-	76.4±1.8	84.3±2.6
F11 A	-	-	400	-	-	72.3±1.6	81.6±2.6
F12 A	-	-	500	-	-	66.2±1.2	79.6±2.4
F13A	25	0.5	200	25 °	5	71.3±2.2	83.8±1.6
F14 A	-	-	-	37 °	-	76.2±1.8	92.6±1.6
F15 A	-	-	-	45 °	-	78.2±1.8	78.2±1.6
F16 A	25	0.5	200	37 °	1	75.4±1.6	76.8±1.2
F17 A	-	-	-	-	5	82.8±1.2	83.5±1.8
F18 A	-	-	-	-	10	85.4±0.9	95.2±1.4
F19 A	-	-	-	-	20	78.5±1.2	83.5±1.8

Evaluation Parameters

The prepared microspheres of Azathioprine drug were evaluated for particle size, drug entrapment efficiency, percent yield, invitro release studies and stability studies.

Particle size analysis

The particle size of microspheres was determined using optical microscopy method. Particle size of all the batches of azathioprine microspheres and budesonide microspheres sample was measured with an optical micrometre fitted with a calibrated eye piece. Approximately 200 particles were counted for particle size using a calibrated optical microscope. All readings are average of three trials ± SD ⁹.

Drug entrapment efficiency

Drug entrapment efficiency Drug entrapment efficiency of Azathioprine microspheres was performed by accurately weighing 100 mg of drug equivalent microspheres and suspended in 100 ml of 7.4 pH phosphate buffer and it was kept on a side for 24 hours. Then, it was stirred for 15 mins and filtered. After suitable dilution, azathioprine in the filtrate was analyzed spectrophotometrically at 280 nm using U.V. Spectrophotometer ¹⁰.

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 10$$

Percentage yield

The prepared azathioprine microspheres with a size range of 60-90 µm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres ¹¹.

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \times 10$$

Scanning electron microscopy analysis (SEM)

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, jeol, Japan). Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 3.0 KV during scanning. Microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology.

In vitro drug release study

Test were carried out for azathioprine microspheres using separate USP apparatus II (paddle) and the medium was Simulated gastric fluid, Simulated intestinal fluid and

simulated colonic fluid, individually. Quantity of dissolution medium was 900 mL. The speed of paddle was 50 rpm and temperature of dissolution medium was 37.5°C. The accurately weighed azathioprine microspheres were placed in the dissolution medium and apparatus was run. At intervals of 2, 5, 8, 12, 16, 20 and 24 hours, 5 mL aliquots were withdrawn and replacement was made each time with 5 mL of fresh dissolution medium⁶. Each 5 mL sample was filtered through Whatman filter paper no. 41 and diluted up to 50 mL with respective dissolution medium. Then absorbance was measured at 280 nm¹².

Stability Study

Stability studies were carried out at $5 \pm 3^\circ\text{C}$, $25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH (room temperature) and $40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH for the optimized formulation F18 A and F18 B for 3 months. The samples were withdrawn after predetermined period of 1 month, 2 month and 3 month. The samples were analyzed for particle size and % residual drugs¹³.

Drug Release Kinetics

Zero order release rate kinetics¹⁴

To study the zero order release kinetics the release rate data are fitted to the following equation $F = K_0t$

Here, F is the fraction of drug release

K_0 is the rate constant

T is the release time

First order model

This model has also been used to describe absorption and elimination of drug, the release of the drug which followed first order kinetic can be expressed by the equation

$$\log C = \log C_0 - kt/2.303$$

Where, C_0 is the initial concentration of drug

K is the first order rate constant

t = is the time

Higuchi release model

To study the Higuchi release kinetics, the release rate data was fitted to the following equation

$$F = K_h t^{1/2}$$

Where, F is the amount of the drug release

K_h is the release time

t is the release time

Korsmeyer and peppas model

The release rate data were fitted to the following equation,

$$M_t/M_\infty = K_m t^n$$

M_t/M_∞ is the fraction of drug release

K_m is the release constant t is the release time.

3. RESULTS AND DISCUSSION

Evaluation parameters of microsphere

Microspheres of azathioprine were prepared successfully by solvent evaporation method. The various parameters in the production of microspheres were evaluated and reported in Table 1. The particle size study was found to be between 75.4 ± 1.6 to 85.4 ± 0.9 of F16 A - F19 A. The particle size of microspheres increased with increases in the polymer concentration. The drug entrapment efficiency was found to be range of 76.8 ± 1.2 to 95.2 ± 2.1 . Initially the entrapment efficiency microspheres is increased with increasing the polymer concentration but after optimum condition entrapment efficiency is decreased due to less amount of drug available as compare to polymer for entrapment. The Percent yield was found to be between 71.56 ± 1.4 to 84.23 ± 2.5 . The shape and surface characteristics were determined by scanning electron microscopy and report shows in Fig 1.

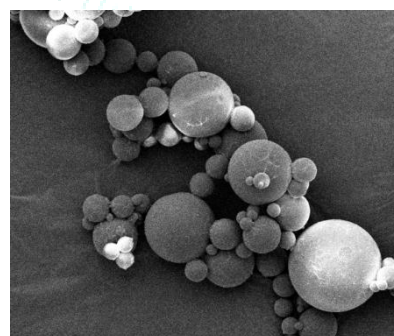


Figure 1: SEM image of optimized formulation F18 A

Dissolution Studies

All the F16 A - F19 A formulations of azathioprine microspheres are subjected to dissolution studies. Dissolution is carried out in USP type II apparatus at 50 rpm in the volume of 900 mL dissolution medium was simulated gastric fluid, simulated intestinal fluid and simulated colonic fluid for 24 hours. The results are shown in Table 2 and Fig.2.

Table 2: Release study of Formulation F16 A- F19 A

Simulated Media (pH)	Time in Hours	F16 A	F17 A	F18 A	F19 A
		Cumulative % Drug release			
SIF 1.2	1	15.42	10.17	4.23	11.40
	2	26.30	18.58	8.96	20.86
SIF 6.8	5	40.22	36.56	12.64	34.70
SCF 7.4	8	60.32	68.84	56.20	62.17
	12	68.46	72.08	68.00	73.32
	16	75.37	89.40	86.80	84.39
	20	83.92	92.82	95.50	90.46
	24	96.70	98.08	99.01	97.20

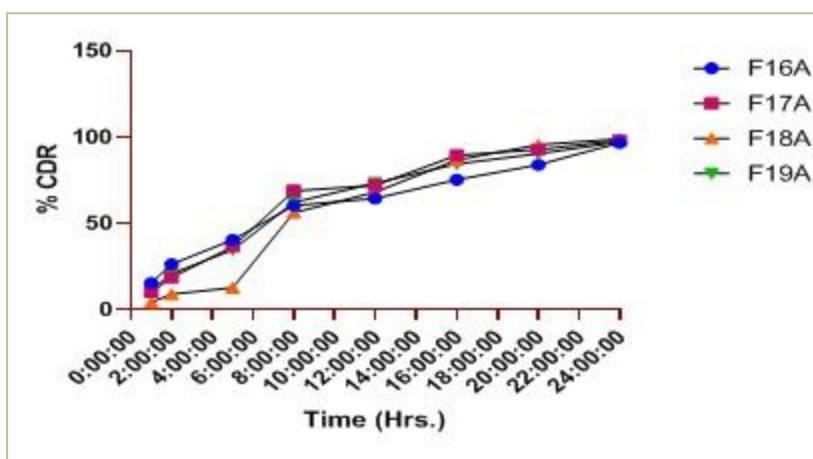


Figure 2: Release study of formulation F16 A - F19 A

Drug release studies shows that F18 A shows good release behavior in colon and restricts release in stomach and intestine as compare to F16 A, F17 A, and F19 A. Because dextrin is degraded by the enzymes presented in the colonic region hence this study confirms that for colon targeting by dextrin can act as good carrier.

Stability study of formulation F 18 A confirms that microsphere are more stable in refrigerator condition at $5 \pm 3^\circ$ and there was no significant change occurs in particle size and % residual drug content of F18 A.

The Fig. 2 indicates F18 formulation shows better drug release when compared with other formulations, and followed by the first order kinetics. The mechanism of release F18 A formulation as shown in Fig 3-6.

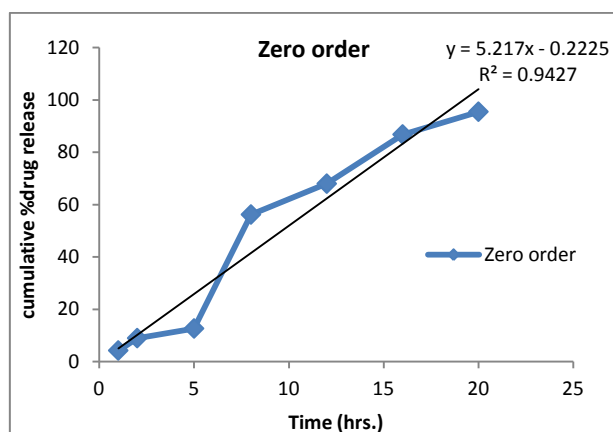


Figure 3: Zero order release kinetics of F18 A

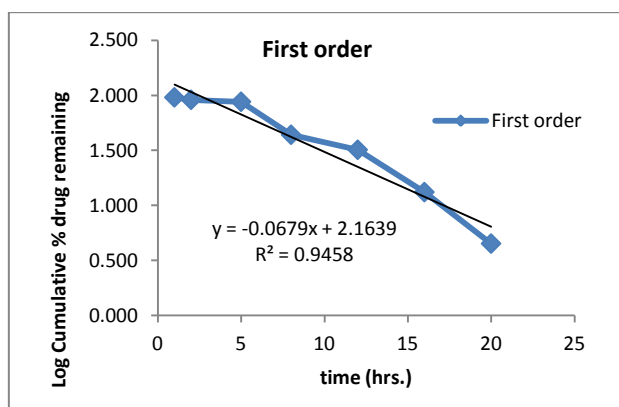


Figure 4: First order release kinetics of F18 A

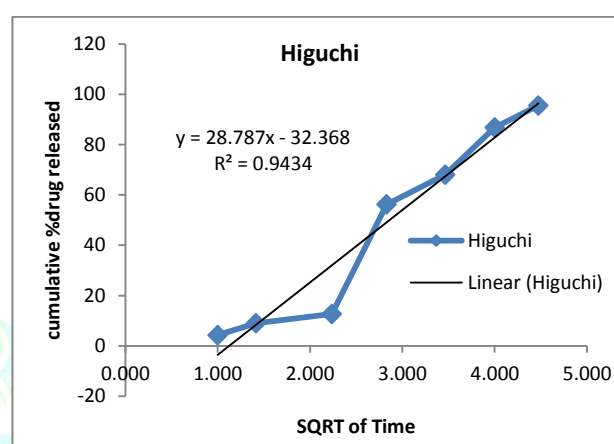


Figure 5: Higuchi order release kinetics of F18 A

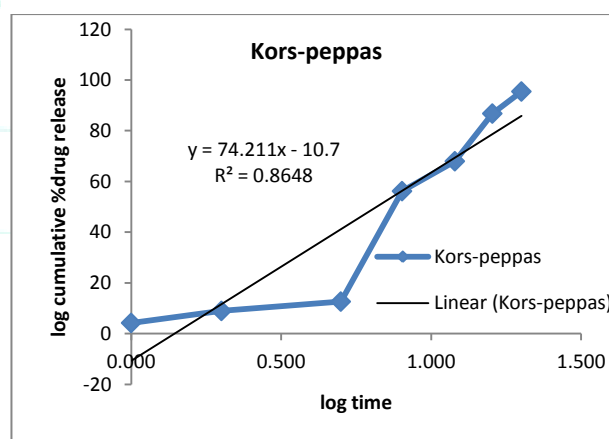


Figure 6: Korsmeyer - Peppas release kinetics of F18 A

4. CONCLUSION

Drug release studies showed that Dextrin based azathioprine microspheres shows good release behaviour in colon and restricts release in stomach and intestine. This study confirms that for colon specific targeting, dextrin can act as good carrier which delivers the more amount of drug specifically in colon.

REFERENCES

1. Kinget R, Kalala W, Vervoort L, Mooter G, Colonic drug targeting. *J Drug Target*; 1998; 6:129-149.
2. Watts PJ, Illum L, Colonic drug delivery. *Drug Dev. Ind Pharm*;1997; 23:893-913.
3. Yang L, Chu JS, Fix JA, Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. *Int J Pharm*; 2002; 235:1- 15.
4. Halsas M., Penttinen T, Veski, P, Jurjenson, H, Marvola, M, Time controlled release pseudoephedrine tablets: bioavailability and in vitro/in vivo correlations. *Pharmazie*;2001; 56:718-723.
5. Halsas M, Hietala, J, Veski, P, Jurjenson H, Marvola M, Morning versus evening dosing of ibuprofen using conventional and time controlled release formulations. *Int J Pharm*;1999; 189: 179-185.
6. Priyanka S, Chaudhari K, Slunkhe S, Amrutkar P, chaudhari SV and Oswal SJ, formulation and development of colon specific drug delivery using dextrin, *International Journal of Pharma and Bio Sciences*,2012; 3(1):269-276 .
7. Zou MJ, Cheng G, The oral colon specific drug delivery system. *Shenyang Yaok eDaxu eXuebao*; 2001;18: 376-380.
8. Joseph S and Shaji S, Formulation And Evaluation Of Losartan Microspheres, *International Journal Of Research In Pharmacy And Chemistry*, 2015; 5(4):555-563.
9. Srivastava AK, Ridhurkar DN and Wadha S. Floating microspheres of cimetidine: formulation, characterization and in vitro evaluation. *Acta Pharm Res*. 2005; 277-285.
10. Sandeep S. Formulation and evaluation of colon targeted drug delivery of an anti-amoebic drug. *Int J Pharm Innov*. 2012; 2(2):138-152.
11. Chandiran IS, Sivakumar T, Kumar BP. Preparation and evaluation of aceclofenac loaded biodegradable microspheres. *Int J Pharm Biomed Res*. 2010; 1(1)19e23
12. Kishor SK And Mohan VK, Formulation And In-Vitro Evaluation Of Dextrin Matrix Tablet Of Ibuprofen For Colon Specific Drug Delivery, *Pak. J. Pharm. Sci.*, 2008; 21(1):17-20.
13. Pai RV, Jain RR, Bannaliker AS, Menon MD, Development and Evaluation of Chitosan Microparticles Based Dry Powder Inhalation Formulations of Rifampicin and Rifabutin, *Journal of Aerosol Medicine and Pulmonary Drug Delivery* , 2015; 28:1-17.
14. Gautam S, Mahaveer S, Review: In-Vitro Drug Release Characterization Models, *International Journal of Pharmaceutical Studies and Research*, (2011; 2(1):77-84.
15. Momin M and Pundarikakshudu K., In vitro studies on guar gum based formulation for the colon targeted delivery of Sennosides. *J. Pharm. Pharm. Sci.*, 2004; 7(3): 325-331.
14. Indian Pharmacopoeia, Government of India Ministry of Health and Family Welfare, published by Controller of publication, Delhi , (1996).Vol. II, 736.
15. Lachman L, Herbert A, Liberman J, The Theory and Practice of Industrial Pharmacy, Varghese publishing House, Hind Rajasthan Building Dadar Mumbai-400001, (2004)3 rd Edn., 318.

